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Bird repellents: development of avian-specific tear gases for resolution of human–wildlife conflicts

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Abstract

The use of bird repellents may be required when human activities place birds in danger, e.g., hazardous waste sites, or when birds cause damage to crops, architectural structures, or are a source of zoonotic disease. Typical protective measures to keep birds away from areas include exclusion by use of netting, hazing (i.e., scaring tactics) and chemical repellents. Birds can rapidly habituate to visual and auditory hazing if the use of these tactics falls into a predictable pattern, or if the sign stimuli are not coupled with a salient aversive reinforcing stimulus. Chemical repellents are typically used to render a resource unpalatable and, as a consequence, create a disincentive for a bird to visit a particular area. Methyl anthranilate (MA) is a potent avian chemosensory irritant. In this paper, we explore the possibility of employing MA aerosols as a bird deterrent strategy. We determine the behavioral response of starlings (*Sturnus vulgaris*) to each of three aerosols: water or yucca extract (controls) and ReJeX-iT TP-40™ (a 40% MA solution), and found that starlings were irritated by exposure to the MA aerosol. Moreover, starlings did not habituate to repeated exposure to MA aerosols. We determined in the laboratory that the starlings' threshold for irritation to a formulated aerosol was 8% MA.

1. Introduction

Human–wildlife conflicts can occur when industrial or agricultural activity negatively affects the well-being of wildlife populations, when the activity of wildlife results in crop or architectural damage, or when the proximity of wildlife to humans results in disease or parasite transmission to humans or their domestic animals (Mason and Clark, 1992). Repellents are used in these circumstances to move wildlife away from the area where the conflicts occur.

Typical protective measures to keep birds away from areas include exclusion by use of netting, hazing and chemical repellents (Hyngstrom et al., 1994). Depending on circumstances, exclusionary netting and its required support structures may not be economically or logistically feasible, e.g., when large areas need to be protected or when intact liners or chemical barriers are used to contain hazardous materials. Hazing techniques rely on the use of auditory and visual devices to scare birds away from an area, e.g., bird distress calls, pyrotechnics, propane exploders, flashing lights, effigies of humans or predators and flagging. However, birds can rapidly habituate to these tactics if the use of such devices falls into a predictable pattern (Allen, 1990), or if the stimuli are not coupled with additional salient aversive reinforcing stimuli (Lehner, 1996). Thus, hazing requires diligence on the part of managers to maintain the novelty and effectiveness of the stimuli. Chemical repellents, both primary and secondary (Rogers, 1980), are used to render a resource unpalatable, e.g., food or water and, as a consequence, repellents remove the incentive for birds to visit the area where the protected resource occurs (Mason and Clark, 1992; Dolbeer et al., 1994).

Primary chemical repellents have odor, taste, or irritating qualities that result in a congenital, unlearned avoidance of the compounds. Plants have exploited the sensory systems of animals as a basis of their chemical defenses (Harborne, 1982). For example, capsaicin is the hot-pungent chemical in chili peppers and mammalian seed predators are acutely sensitive to this compound (Clark, 1998). Although birds are insensitive to capsaicin as an irritant (Szolcsanyi et al., 1986), they are sensitive to

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other naturally-derived compounds (Mason et al., 1992; Shah et al., 1992). To a bird, the principal ingredient of grape flavoring, methyl anthranilate (MA), is a potent irritant (Kare, 1961; Mason et al., 1989; Clark, 1996).

Chemical repellents have typically been used as feeding deterrents against birds in agricultural and horticultural contexts where birds are the cause of damage to crops and urban landscapes. Chemical repellents have rarely been used as a protective tactic to save birds in industrial waste water settings (however, see Clark et al., 1998), primarily because even sampling of small amounts of hazardous wastes can prove to be fatal. There is a clear need to develop chemical repellents that do not require ingestion to be effective, that are themselves environmentally safe, that are resistant to habituation and that can be economically employed to protect large areas. This paper explores the feasibility of developing such an avian repellent.

The nociceptive system that mediates the sensation of orally presented irritants also innervates the mucosae of the nose and eyes. This attribute has been exploited in the use of ortho-chlorobenzylidene malononitrile (CS) and chloroacetophenone (CN) tear gases for use in human crowd control (Anderson et al., 1996; Yih, 1995). Thus, by analogy, it is arguable that the bird-specific irritant, MA, may be useful as an area repellent to target birds in flight, prior to their contact with contaminated water. Herein we describe a behavioral assay relating to the responsiveness of European starlings (*Sturnus vulgaris*) to aerosols as a function of MA concentration. We also investigate the short-term behavioral habituation response of starlings to aerosol exposure.

2. Materials and methods

2.1. Test subjects

Adult European Starlings were decoy-trapped at Sandusky, Ohio and transported to the National Wildlife Research Center, Fort Collins, Colorado, in accordance with institutional animal care and use guidelines. Birds were housed in individual cages (38 × 25 × 28 cm), provided food and water *ad libitum* and were maintained on a seasonally shifting photoperiod (October February, 40 N) at constant air temperature, 19 C.

2.2. Test stimuli

Test stimuli were presented as pulsed aerosols generated from liquid reservoirs. Water was the control stimulus. ReJeX-iT TP-40^{TM}, a commercially available proprietary formulation of MA (40% vol./vol.), was the test stimulus (RJ Advantage, Inc., Cincinnati, Ohio). Yucca extract (New Waste Concepts, Perrysburg, Ohio) was also tested as a control and was subsequently used as an emulsifier to achieve the desired aqueous phase dilutions of TP-40. In pilot testing, yucca produced stable colloids when mixed with water-insoluble compounds and it was incorporated in these experiments to further test it as a non-irritating solvent.

2.3. Test chamber

An acrylic test chamber (183 × 60 × 48 cm) was equipped with four Bete (Model XA SR, Greenfield, Massachusetts) air atomizing nozzles placed at one end of the chamber and positioned at a height of 34 cm. (Figure 1). Droplet size was approximately 70 μm according to manufacturer's specifications. The normal state of the system allowed pressurized air to be delivered through the nozzles, providing a source of fresh, unfiltered air into the chamber. Solenoid valves allowed controlled releases of test and control liquids into the nozzle heads to generate aerosol pulses. Two separate nozzles were designated for control and test aerosols.

By placing water-sensitive paper targets throughout the chamber, it was determined that relatively even droplet coverage was achieved in the range of 120 to 180 cm downwind from the nozzles. To ensure uniform aerosol exposure to the birds, they were restricted to the last 60 cm section of the chamber by a wire screen (0.5 × 0.5 cm). Air and aerosols were drawn through two vents in the rear of the chamber by a 12 V, 7.5 cm. fan. Clearance time for an aerosol pulse in the chamber was approximately 4 min. Two 50 ml drinking tubes attached to the screen provided drinking water for the bird during a trial; placement of the tubes was configured to minimize contamination by aerosol droplets.

2.4. Behavioral assay

The test of an aerosol treatment consisted of five trials involving one bird each. Trials were monitored and recorded with video equipment. Behavioral responses, operationally defined as indicating irritation, were: bill wiping, gagging/vomiting, head shaking, piloerection and quick-preening, i.e., bouts shorter than two seconds in duration. The frequency of these behaviors was used as an index of irritation. Video recordings were coded and set aside for subsequent analysis. To control for potential observer bias, the person scoring the video records for irritation behaviors was blind to the identity of the test stimuli.

Irritation behavior frequencies were summed in the minute prior to and in the minute after the onset of a stimulus presentation. Irritation responses were then quantified as the difference between these pre- and post-presentation frequencies. Each trial was 75 min. The first 10 min served as an acclimation period during which no aerosols were presented. During the remaining 65 min, aerosols ($n = 5$ presentations/bird) were presented

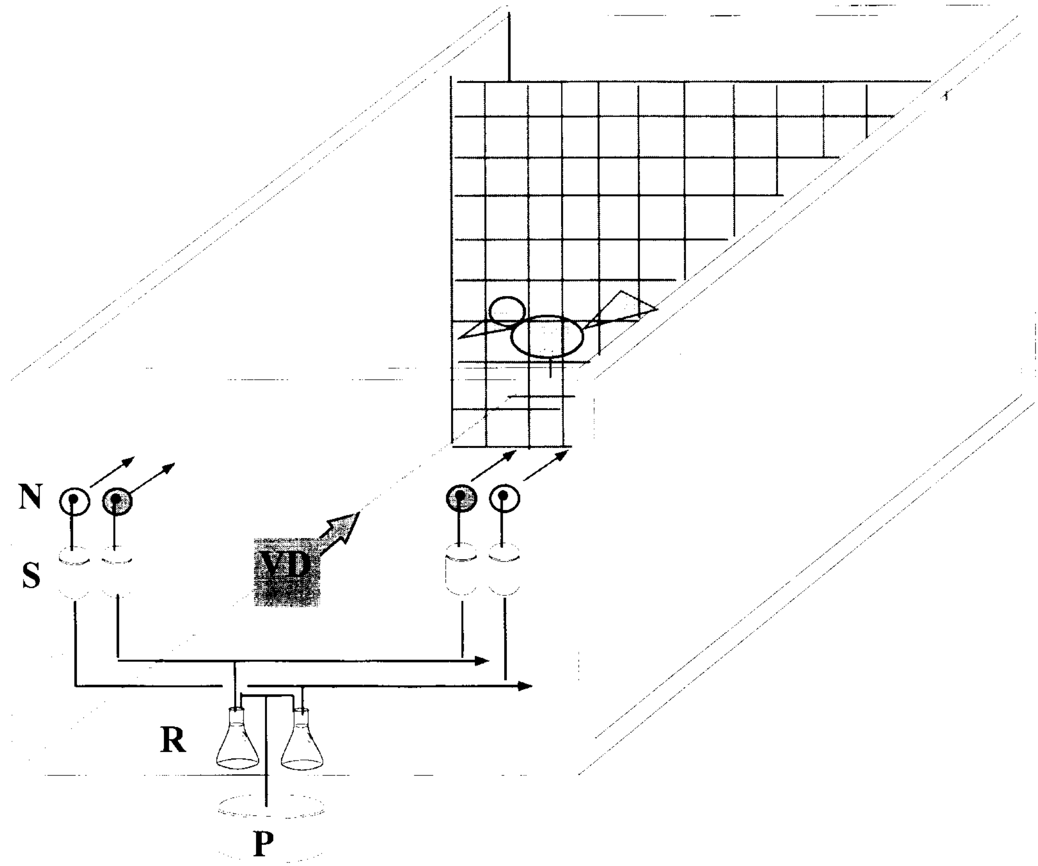

Fig. 1. A schematic diagram of the aerosol test chamber. P is the pressurized pump. R is the reservoir of test liquids drawn by vacuum into the aerosol nozzles (N) when the solenoid (S) is activated. VD is the video camera recording bird behavior.

(0.5 min) with randomly determined interstimulus intervals ranging from 4 to 8 min. For each of these presentations, the designation of control (water) or test aerosol was randomly assigned.

2.5. *Experiment 1. Tests of aerosol effects and habituation*

The objective of this experiment was to determine the behavioral response of starlings to each of three aerosol types: water (control), TP-40 (a MA-containing formulated bird repellent) and yucca extract (an emulsifier). Data were analyzed using a three factor mixed effects analysis of variance (ANOVA), where the between-measures effect was treatment, and the within-measures effects were time of stimulus presentation and subjects (5 subjects, nested within treatment).

Post hoc analyses were threefold:

1. we determined if an aerosol treatment was irritating upon initial exposure by testing whether the intercept (b_1) of the regression between response and time of stimulus presentation was equal to zero (i.e., indicative of no response to the stimulus);
2. we looked for evidence of sensory adaptation (an increase or decrease in response under repeated aerosol exposure) by testing whether the slope of the regression line (b_0) was equal to zero and;
3. we used Tukey's multiple comparisons test to compare treatment means where significant differences were found by the ANOVA.

2.6. *Experiment 2. Concentration response relationships*

The objectives of this experiment were to develop a concentration–response curve for starlings to MA aerosols and to determine their threshold detection level. Threshold was defined as the minimum concentration that elicited an irritation response significantly greater than the water control. The concentration–response curve was fitted to the median irritation index frequencies for each concentration. Concentrations of MA contained in the test formulations were: 16%, 8%, 2% and 1%. To obtain these MA concentrations in a water solution, 3 × yucca extract (75% vol/vol) (New Waste Concepts, Inc., Toledo, OH) was added. Data from TP-40 (40% MA) and water (0% MA) trials from Experiment 1 were included with results from this experiment. Tukey's multiple comparisons test was used in a post hoc analysis to compare treatment medians.

3. Results

3.1. Experiment 1. Tests of aerosol effects and habituation

Average levels of response elicited by the three treatments were not equal ($F=60.63$, $P<0.0001$). Tukey's multiple comparisons test showed that starlings were more agitated during TP-40 aerosol presentation, relative to presentations of the two control aerosols, water and yucca (Figure 2, inset).

The initial response to TP-40 was high and significantly different from the null hypothesis of no response (Table 1). Although the starlings' initial response to yucca aerosols was low, and 5.5 times less than the level of responsiveness to TP-40, it was still higher than the null condition of no response at all Table 1. The effect of TP-40 on starlings was marginally sustained with no statistical evidence of sensory adaptation to repeated episodic exposure, i.e., response did not change as a function of multiple exposure over time, $P=0.07$, even though there appeared to be some tendency for adaptation Fig. 2. The lack of statistical habituation to exposure to TP-40 is primarily due to the high variability in responsiveness observed for this stimulus. These observations are not unusual. Variability can be attributed to variations in intrinsic sensitivity across individuals (Clark, 1998). Unlike the trials with TP-40, the downward-sloping regression line for yucca aerosols (b_0, $P=0.01$) is evidence that starlings soon adapted to the stimulus after repeated exposure. Ultimately these birds converged to an average level of responsiveness similar to that observed for the water control (Figure 2, inset).

We infer that the initial response to TP-40 and Yucca was most likely due to the physiological effects of these aerosols, because the initial responsiveness of starlings to the water control did not differ from zero, nor did the response to water change as a function of repeated exposure to the control Table 1.

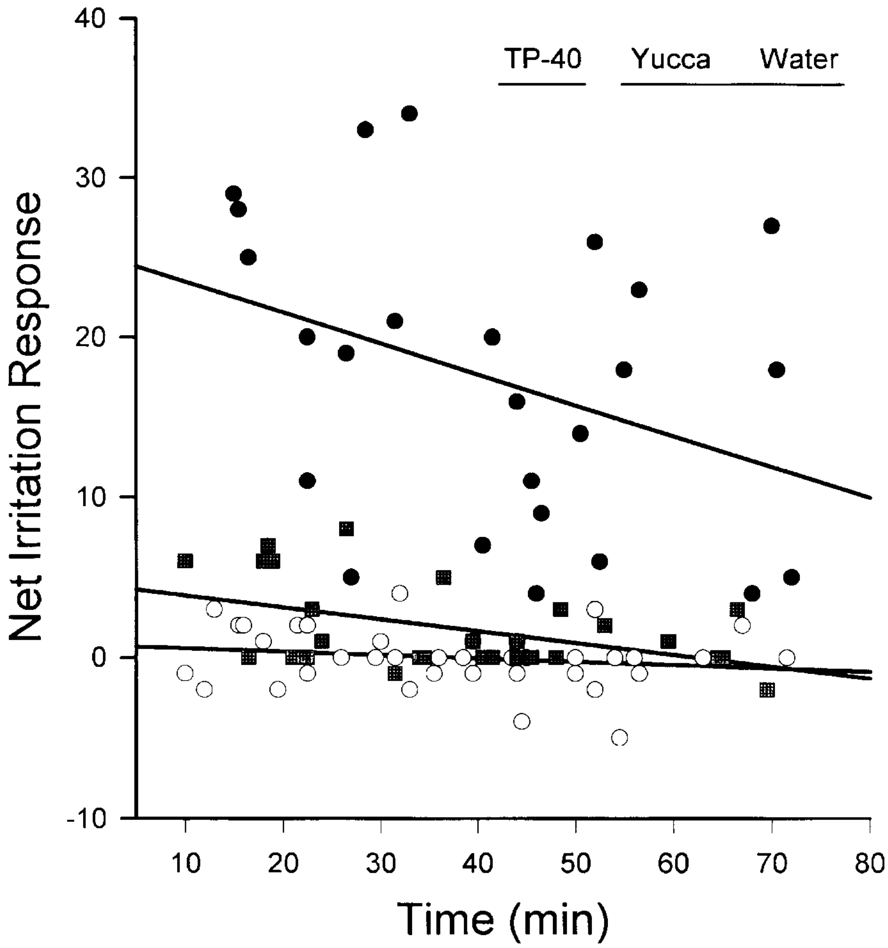

Fig. 2. The frequency of irritations behaviors recorded for starlings during aerosol presentation, adjusted for pre-aerosol behavioral activity as a function of time within the trial. Aerosol presentations consisted of one of three treatments for each cohort of birds ($n=5$/cohort): TP-40 (solid circles), yucca extract (shaded squares) and water (open circles). Lines depict fitted regressions (see text). The inset depicts the post hoc test for similarity among mean responsiventess to the three aerosol types. Connected lines indicate similarity in response level ($P>0.05$).

Table 1
Regression parameters for starlings' responsiveness to aerosols[a].

	TP-40	Yucca	Water
$b_1 \pm$ SEM	25.4 ± 4.6	4.5 ± 1.1	-1.1 ± 1.4
$b_0 \pm$ SEM	-0.2 ± 0.1	-0.1 ± 0.0	0.0 ± 0.0
R^2	13.5	21.5	3.2
df	24	27	16
$H_0{:}b_1 = 0$	$t = 5.5$, $P < 0.001$	$t = 4.0$, $P < 0.001$	$t = -0.8$, $P = 0.$
$H_0{:}b_0 = 0$	$t = -1.9$, $p = 0.07$	$t = -2.7$, $P = 0.01$	$t = 0.7$, $P = 0.$

[a] b_1 is the intercept of the linear regression, $I = b_0T + b_1$, where I is the net irritation response (adjusted for pre-exposure activity), T is the time of presentation of the aerosol stimulus, and b_0 is the slope. SEM is one standard error of the mean. R^2 is the total variance explained by the model, ad df is the degrees of freedom. $H_0{:}b_1 = 0$ is a test of the hypothesis that there is no initial response to the aerosol. $H_0{:}b_0 = 0$ is the test of the hypothesis that the response does not change over time and, by implication, exposure history.

3.2. *Experiment 2. Concentration response relationships*

Levels of response elicited by the six MA concentrations were not equal ($F = 7.53$, $P = 0.0002$). The level of agitation expressed by starlings increased as a function of MA concentration presented in the aerosol (Figure 3) and can be expressed by the relationship $I = b_1C^{b_0}$, where I is the median frequency of the net irritation response (adjusted for pre-exposure activity), b_1 is the intercept (2.8 ± 0.5), b_0 is the slope (0.6 ± 0.1) and C is the concentration of methyl anthranilate in the aerosol ($R^2 = 98.0$, $P < 0.001$).

Post hoc comparisons indicated that 8% MA was the threshold detection level for starlings (Figure 3, inset), as concentrations of 8% or greater elicited an agitation response greater than that observed for controls ($P < 0.001$). While the concentration–response relationship indicates higher responsiveness at higher MA concentrations, post hoc comparisons of response indicate that responsiveness is similar at 8, 16 and 40% MA ($P = 0.08$).

4. Discussion

The development of aerosol delivery strategies for avian repellents is analogous to the use of tear gas for human crowd control. For mammals, the basis for the potency of tear gas is the direct contact of the active agent, e.g., CS or CN gas, with chemical nociceptors in the mucosae of the eye, nose, mouth or respiratory tract. Physiological effects may include lacrimation with blepharospasm, corneal redness and edema, sensations of burning and pain, laryngospasm, salivation and vomiting (Anderson et al., 1996; Yih, 1995). Tear gas is principally used to elicit a change in behavior, i.e., to promote avoidance of an area, based on severe sensory irritation. Theoretically, the use of avian primary repellents as aerosols employs a similar strategy. To affect behavior, a sensory irritant must be applied in the airspace over a protected resource, e.g., a waste water impoundment, and must be concentrated in such a way as to maximize the likelihood of targeting incoming birds (Mason and Clark, 1992). While exposing birds to irritant aerosols is not a benign management strategy, it is a less severe alternative than allowing a bird to come into contact with a potentially lethal exposure to contaminated water. Transient exposure to irritants should alter behavior, but not produce long lasting physiologic effects. The bird repellent, methyl anthranilate, meets these criteria.

Our results are not endorsements of particular application rates. Rather, they represent a first step in developing management tools based on repellent aerosols. For example, in the field, a successful aerosol delivery strategy must take into account factors affecting aerosol plume behavior. Standard plume monitoring involves measurements of wind speed and direction, the amount of effluent released and the source height and initial velocity of the plume (Neiburger, 1973). For large-scale plume releases, e.g., industrial smokestacks, knowledge of weather conditions and local topography also contributes to monitoring efforts (Briggs, 1969). Software packages that model aerosol behavior have been developed for use as an aid in hazard assessment of accidental spills and emissions of potentially toxic substances and Clark and Shah (1992) have applied these models to predict olfactory-mediated foraging behavior in Leach's Storm Petrels (*Oceanodroma leucophrys*). Plume modeling efforts are critical to bird hazing operations that wish to employ repellent aerosols, because they can aid in the optimal positioning of the aerosol distribution devices. Underlying this effort is the need to establish the reaction threshold of the target species, both in terms of the concentration of active ingredient in an aerosol particle, and in terms of the particle density in the atmosphere.

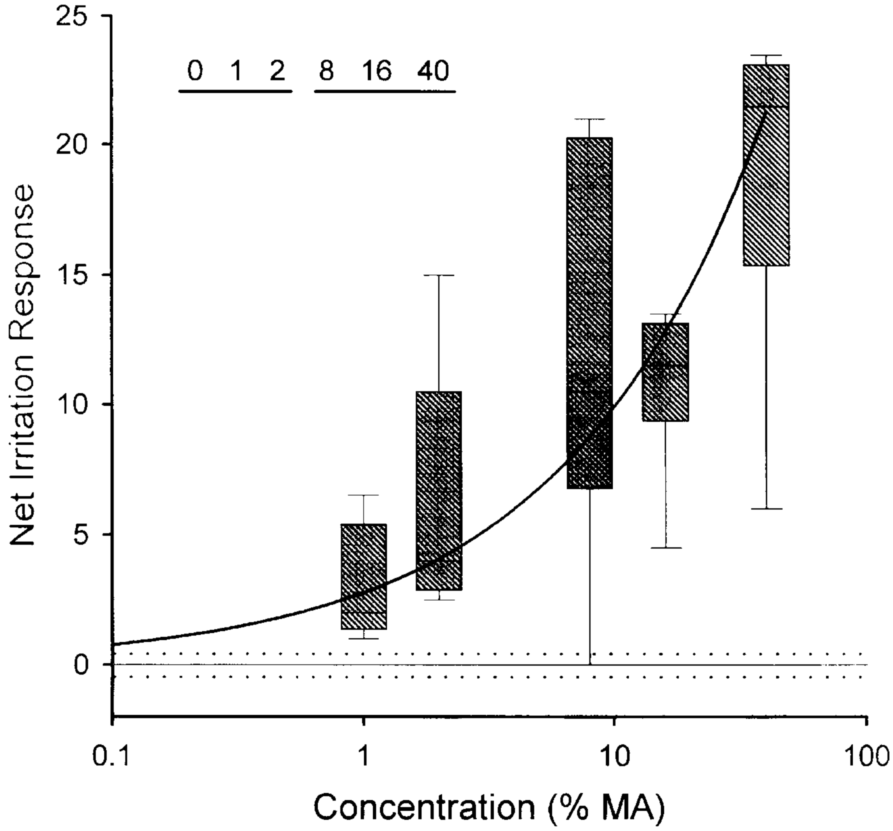

Fig. 3. The frequency of irritation behavior recorded for starlings adjusted for pre-aerosol behavioral activity as a function of methyl anthranilate concentration. The horizontal lines indicated median responsiveness to the aerosol. The vertical bars depict the 75th percentile and the capped lines depict the 95th percentiles about the median response. The curved line is a least squares fit to the median responsiveness. The horizontal reference line (solid) is the median responsiveness of birds presented with a water control with the 95% confidence intervals (horizontal dotted lines). The inset depicts the post hoc comparison of responsiveness for cohorts of starlings tested at each concentration. Concentrations connection by lines are similar at the $P > 0.05$ level.

This study is the first investigation of the threshold sensitivities of birds to repellents delivered in aerosol form.

The effective concentration for an aerosolized irritant of 8%, as determined in this study, seems high relative to previous work with MA. In the laboratory, concentrations of 0.5–2.0% are effective repellent concentrations in feeding trials, while 0.1–1.0% effectively repelled birds from consumption of treated water during drinking trials (Mason et al., 1989; Clark et al., 1991). These differences in the effective MA concentration presumably reflect differences in target specificity, location of effect, or application strategy. For example, when exposing birds to irritants via aerosols, repellency is mediated by contact with trigeminal nerve endings in the cornea, an area of approximately 26 mm^2 in European starlings. This represents a very small target area relative to the volume of the test chamber. In contrast, during feeding and drinking trials the repellent material directly and immediately contacts larger areas of receptors in the buccal cavity. Moreover, a bird's protective nictitating membrane may act as a physical barrier to the aerosol droplets, thereby increasing the concentration or application rate necessary to activate corneal nociceptors.

The responsiveness of a bird to an irritant is a function of the number of molecules accessing receptors. The variance in effective concentration of a repellent reflects the differences in a formulations's capacity to deliver irritating molecules to the receptors. Calculations of the number of molecules accessing the oral, nasal and ocular receptor fields illustrate this point (Dravnieks, 1975). Concentrations of 7–15% vapor saturation (2.7–5.4×10^{13} molecules/unit volume) of a variety of irritants are required to activate trigeminally-mediated responses in pigeons (Michelsen, 1959; Walker et al., 1979). Assuming an activation threshold of 10% vapor saturation, the corresponding molecular concentration for MA would be 3.8×10^{13} at equilibrium. Using this value as a basis of comparison for effective minimum concentration for the repellency of MA, we compared the number of molecules that starlings are exposed to under different delivery tactics. We were able to determine the number of aerosol droplets present in the airspace surrounding a starling per unit time by using chemically sensitive paper targets and counting the number of impacted droplets. Assuming that the primary mediating receptor field for aerosols is the ocular region, we estimated that in an 8% MA solution, the number of molecules impacting the eyes was 3.4×10^{13}, based upon the average particle volume and integrating over a ten second exposure. This value is similar to the average threshold obtained for a broad range of avian chemical irritants (Walker et al., 1979).

Vaporization from the surface of aerosol particles also contributes to the number of molecules in the airspace surrounding a bird. For example, a starling is exposed to 1×10^9 molecules of MA in vapor phase during a ten second aerosol pulse, based upon the average surface area of all aerosol droplets impacting a 1.0 cm^2 area in ten seconds. This value is in addition to the number of molecules contained in the liquid phase of the aerosol particles that directly impact the ocular region. But it is important to note that the vapor concentration by itself is insufficient to act as an irritating stimulus (see also Clark, 1996). Thus, contact irritation is the mode of irritation for aerosols. In contrast, when birds ingest food pellets impregnated with MA, in addition to the number of molecules on the pellet itself that might contact oral receptors, the number of molecules released in vapor phase can quickly exceed the 10% threshold for avian irritancy, i.e., 2.4×10^{19} and 4.5×10^{20} for 1 and 2% treatment levels of food, respectively. The significance of achieving such high concentrations within the oral cavity lies in the ability of these volatilized molecules to travel retro nasally and stimulate nociceptors innervating the nasal capsule (Clark, 1998). For humans, an analogous sensation is the nasal pungency perceived after ingesting horseradish. Similar levels of vapor concentration are achieved in drinking trials for birds ingesting MA solutions. These calculations illustrate the importance of knowing the threshold for irritancy and repellency and of evaluating the efficiency of different modes of delivery to meet this threshold value. Thus, such calculations form the basis of devising formulations and delivery tactics.

5. Wildlife management implications

Traditional bird hazing systems rely on auditory or visual stimuli to scare away flocks and prevent use of an area. Birds can rapidly adapt and learn to ignore stimuli which are not novel (Allen, 1990). Hazing systems that incorporate a strong aversive reinforcer, e.g., a sensory irritant, are therefore more likely to promote and maintain avoidance of a protected resource. The downward-sloping regression line for TP-40 aerosols Fig. 2 indicates the possibility of sensory adaptation over long-term exposure (i.e., 75 min) although in the field, likely exposure time of birds in flight is in the range of 0.5–5 min. To be conservative, a test of the regression slope over the initial 25 min of the trial shows no evidence of sensory adaptation ($H_0{:}b_0 = 0$, $P = 0.87$). It must be pointed out that an irritation response in the laboratory does not directly translate to an avoidance response in the field. Nevertheless, the integration of a chemical repellent such as MA with traditional hazing devices offers a practical solution to the problem of adaptation by increasing the salience of other, non-chemical stimuli.

A direct application of an aerosol bird repellent is for the protection of birds at hazardous waste sites. Waste water impoundments resulting from industrial operations can be a significant contributory risk factor for mortality and morbidity of migratory birds (Kay, 1990). These risk factors are increased when waste water impoundments occur in arid areas where potable water is generally less available. For example, waste water impoundments located in deserts can attract migrating waterfowl to areas not previously documented to be migratory flyways (Allen, 1990). Various treaty agreements set zero tolerance for accidental kills of migratory birds in the United States (e.g., *The Migratory Bird Treaty Act*, 16 U.S.C. 703–711) and enforcement of the regulations places a strong incentive on industry to take measures to prevent the accidental and incidental killing of wildlife.

A second possible application of aerosol repellent strategies is in the context of roost disruption. In many cases, sensory repellents have been employed as topical applications to food or water resources. However, these strategies have little effect where birds gather to roost (Cummings et al., 1995). Large roosting flocks can pose a threat to safety or sanitation when located near airports, landfills, or feed lots (Dolbeer et al., 1993; Gough and Beyer, 1982), or cause nuisance and public health problems (Chick et al., 1980; Dolbeer et al., 1990). Foreseeably, aerosols could be utilized in roost areas to move or disperse large flocks, either alone or in conjunction with other hazing strategies.

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